Supplemental figure legends

Figure S1. Localization of ZYX-1 binding partners in the *zyx-1(gk190)* mutant

Antibodies directed against ALP-1, DEB-1, DYC-1, UIG-1, LIM-8, LIM-9 and SCPL-1 were used to stain the respective proteins in body wall muscles of N2 (wild-type) and *zyx-1(gk190)* worms. No significant modification of the localization of any of the analyzed proteins was detected in the *zyx-1(gk190)* mutant when compared to wild-type. Scale bar 10 µm for all images.

Figure S2. Localization in body wall muscle cells of ZYX-1-GFP and DEB-1 in wild-type and the *atn-1(ok84)* mutant and of DYS-1-GFP and DEB-1 in wild-type.

A-F: Localization of ZYX-1-GFP in body wall muscles of wild-type (A and green in C) and the *atn-1(ok84)* mutant (D and green in F). DEB-1 antibody was used as a positive control for dense bodies organization in wild-type (B and red in C) and *atn-1(ok84)* mutants, (E and red in F). The ZYX-1-GFP and DEB-1 staining partially overlap (merges in C and F). Note that in the *atn-1(ok84)* mutant the DEB-1 and ZYX-1-GFP proteins are still present at dense bodies but their localizations are enlarged compared to wild-type muscles.

G-I: Localization of DYS-1-GFP and DEB-1 in body wall muscles of wild-type worms. DYS-1-GFP in G, DEB-1 in H and the merged image in I shows DYS-1-GFP in green and DEB-1 in red. The DYS-1-GFP protein is abundantly expressed in striated body wall muscles where it localizes in broad bands overlapping actin containing thin filaments, *i.e.* overlapping the whole sarcomere except for the H-zone. The DYS-1-GFP staining seems to be less abundant at the basis of dense bodies and partially overlaps with the DEB-1 staining.

Scale bar 10 µm for all images.

Figure S3. Phalloidin-rhodamin staining of the *dys-1(cx18); hlh-1(cc561)* double mutant and the *dys-1(cx18); hlh-1(cc561ts); zyx-1(gk190)* triple mutant

A-B: Body wall muscles cells analyzed after phalloidin-rhodamin staining of a *dys-1(cx18); hlh-1(cc561)* double mutant (left) and a *dys-1(cx18); hlh-1(cc561ts); zyx-1(gk190)* triple mutant (right). Muscle cells of one quadrant were surrounded by dotted lines. Degenerated muscle cells are labeled in red. The triple mutant presents a diminution by 40% of muscle degeneration when compared to the double mutant (2 degenerated cells *versus* 5). Scale bar 100 µm for both images.

C-D: Enlargement of areas of interest indicated by the white rectangles in A and B. Red asterisks point missing muscle cells surrounded by cells in which actin filaments are visible.

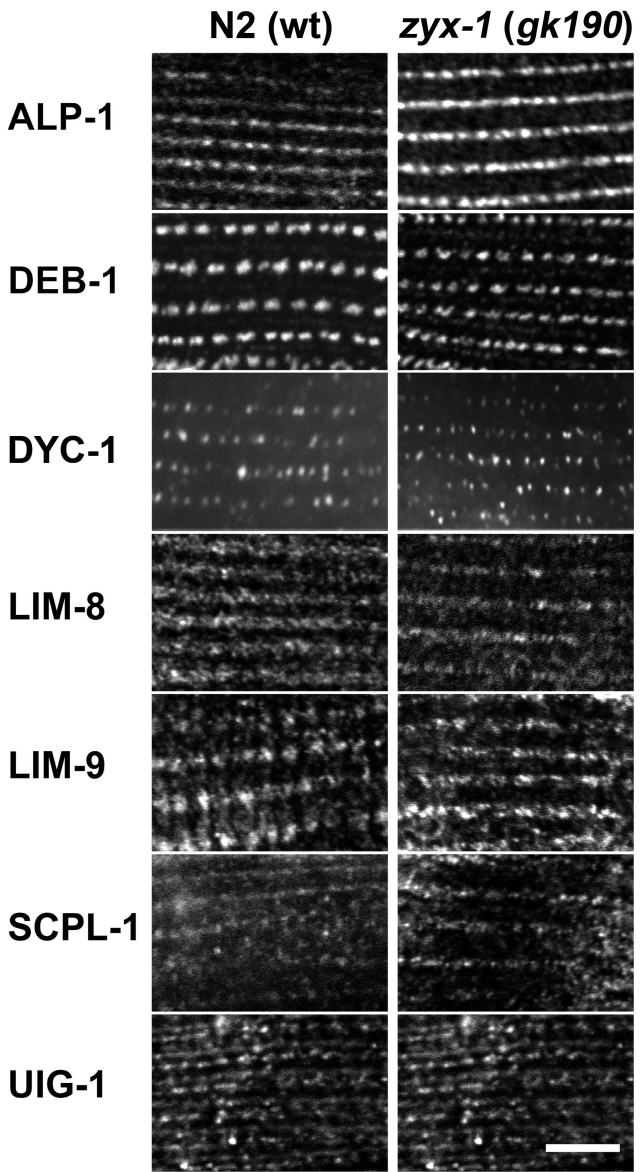
Figure S4. Western blots analysis of ZYX-1-GFP and ZYX-1 proteins

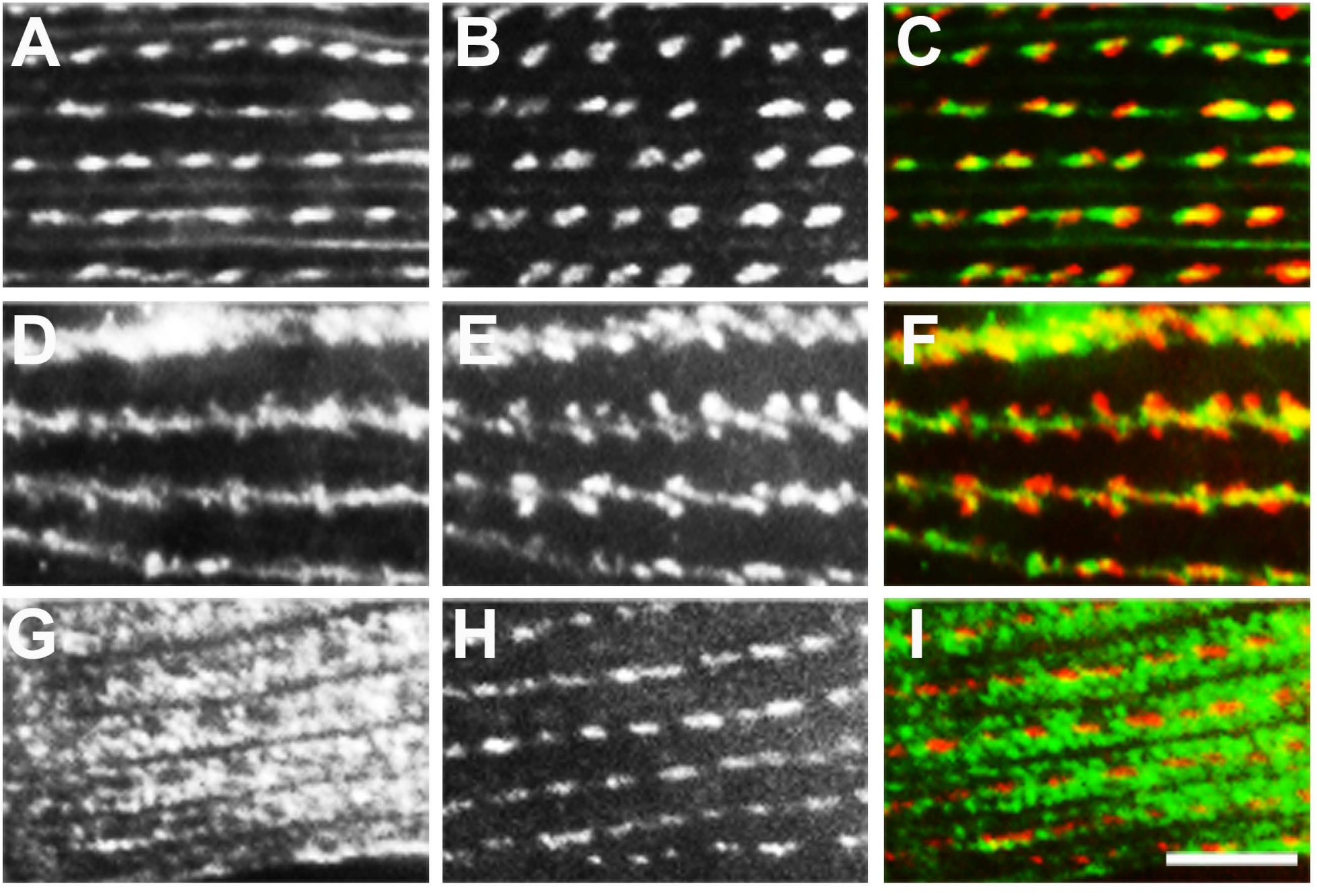
A: Proteins extracted from wild-type worms (lane 1), transgenic worms carrying the pKG001 (lane 2) and pKG106 (lane 3) plasmids were analyzed by Western blot using an antibody directed against GFP. No GFP signal was detected in wild-type worms (lane 1). The bands observed in lane 2 correspond to the predicted proteins encoded by the pKG001 transgene lacking the third LIM domain: 90 kDa for ZYX-1a-GFP (*) and 45 kDa for ZYX-1b-GFP (arrowhead), these proteins are both fused to the 29 kDa GFP protein. The bands observed in lane 3 correspond to the predicted proteins encoded by pKG106: 95 kDa for ZYX-1a-GFP (*) and 50 kDa for ZYX-1b-GFP (arrowhead). In both lanes 2 and 3, the signal of the long ZYX-1a isoform was estimated with Image Lab Biorad Software to correspond to 6% of the total detected ZYX-1-GFP proteins.

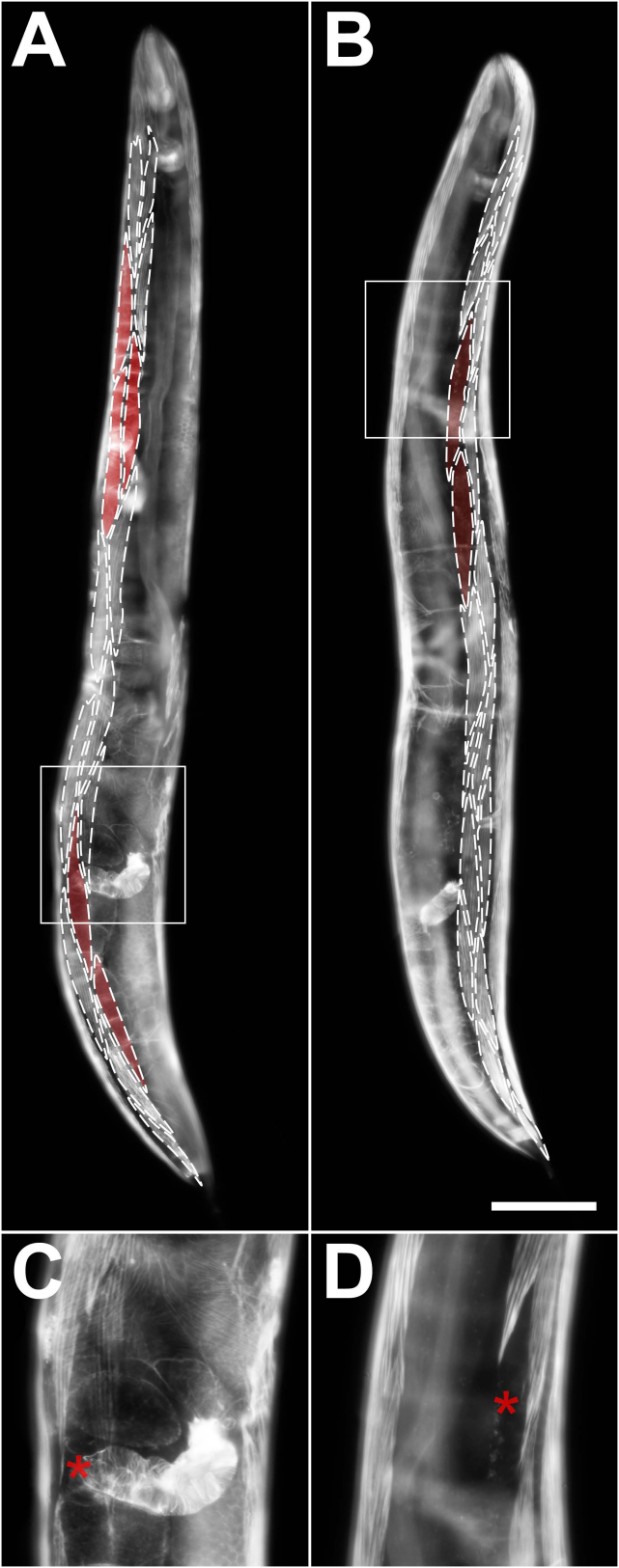
B: Western blot using the monoclonal antibody directed against ZYX-1 were performed on proteins extracted from wild-type and the *zyx-1(gk190)* mutant (lanes 4 and 5). A 23 kDa protein corresponding to the predicted small ZYX-1b isoform is detected in wild-type worms but not in the mutant, along with some weak and non specific bands. We were unable to detect the long ZYX-1a isoform with this antibody although the *zyx-1a* mRNA is present in wild-type (as shown in C). The numbers on the left correspond to the size in kDa of ladder proteins.

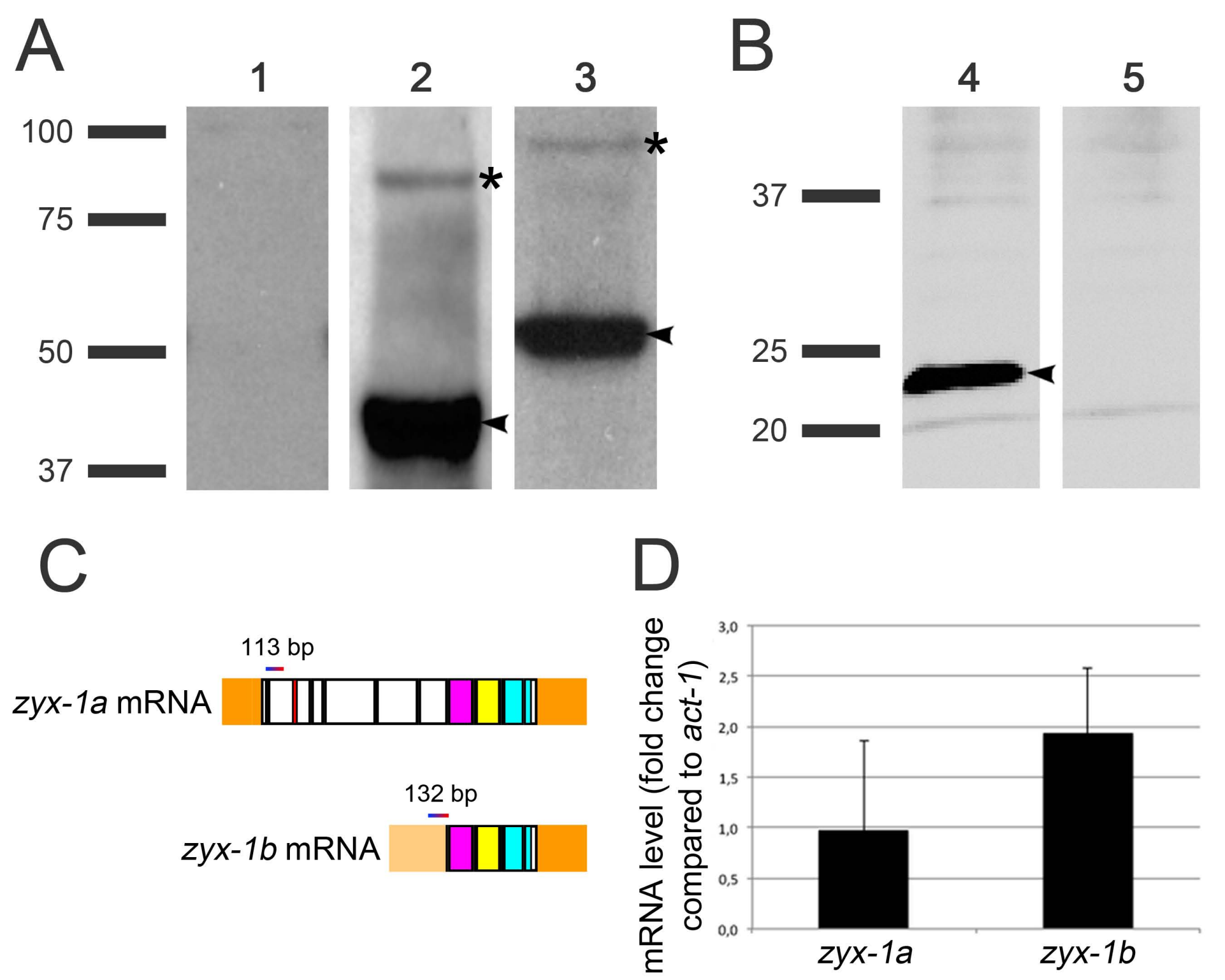
C: *zyx-1a* and *zyx-1b* mRNAs are schematically represented with the specific sequences amplified by RT-qPCR (113 and 132 bp respectively). Note that the forward primer for *zyx-1b* is located in the 5’ untranslated region.

D: *zyx-1a* and *zyx-1b* mRNA levels quantified by RT-qPCR using *act-1* as housekeeping gene control. *The zyx-1b* mRNA is twice more abundant than *zyx-1a* mRNA. Bars correspond to standard deviations between three independent experiments.


N2 (wt)
zyx-1 (gk190)
ALP-1
DEB-1
DYC-1
LIM-8
LIM-9
SCPL-1
UIG-1


A
B
C
D
E
F
G
H
I


A
B
C
D


A
1
2
3
100
75
50
37
*
*
B
4
5
37
25
20
C
113 bp
zyx-1a mRNA
132 bp
zyx-1b mRNA
D
mRNA level (fold change compared to act-1)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
zyx-1a
zyx-1b